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Formulation, Development and Evaluation of Transfersomal Gel of Metronidazole

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ABSTRACT

Metronidazole is an antibiotic that is used to treat a wide variety of infections. It works by stopping the growth of certain bacteria and parasites. This antibiotic treats only certain bacterial and parasitic infections. It may also be used with other medications to treat certain stomach/intestinal ulcers caused by bacteria (H. pylori). A transferosome is the first generation of an elastic liposome prepared from phospholipids and edge activators. An edge activator is often a single-chain surfactant with a high radius of curvature that destabilizes the lipid bilayers of vesicles and increases the deformability of the bilayers, thereby making the vehicle ultra-deformable. The aim of the present study was to investigate the potential of transfersomal gel formulations for transdermal delivery of metronidazole and to evaluate the effect of lipid concentration, ethanol concentration, drug concentration and stirrer time. Characterization of transfersomes performed by vesicle size, surface charge, entrapment efficiency and zeta potential. Characterization of transfersome containing gel performed by the measurement of viscosity, drug content, extrudability study, spreadability and in vitro drug diffusion study. It was found that viscosity of prepared gel was 3560cps, % assay was 98.89±0.45, extrudability was 1156g and spreadibility (g.cm/sec) was found that 11.23(g.cm/sec) respectively. *In vitro* drug release from transfersomes gel was carried out using Franz diffusion cell method and found 85.56% in 12 hr. In first 30 min it was 22.2 % drug release which slightly high. It was due to the release of free drug present in bag after leaching from transfersomes. Drug release from transferosomal gel formulation was found in very sustained and controlled manner. The results were obtained which showed that transfersomal gel was a promising candidate for transdermal delivery with targeted and prolonged release of a drug. It also enhances skin permeation of many drugs.

Keywords: Transfersomal gel, Metronidazole, Antibiotic, Characterization, Franz diffusion cell

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INTRODUCTION

Metronidazole is one of the most preferred drug of choice for intestinal amoebiasis, giardiasis, trichomoniasis, bacterial vagi-nosis, surgical infections and duodenal ulcer associated with Helicobacter pylori infections, etc[1,2]. Metronidazole is slightly soluble in water and is usually about 80% absorbed orally, although absorption in some patients can be much lower[3]. Conventional tablets of metronidazole provide minimal amount of drug for the local action in colon, still resulting in the relief of amoebiasis, but with systemic side effects[4]. The minimum inhibitory concentration and bactericidal concentration of me-tronidazole is 3.1 and 6.3 mg/ml, respectively[5]. Metronidazole dosing regimens often include sets of daily doses extending over periods of several weeks. Since the elimination halftime of metronidazole is 6–8 h; the drug has to be taken 3–4 times per day in order to maintain the desired therapeutic level. Site specific and controlled release dosage forms will prolong the residence time and improve bioavailability[6]. Hence, alternate dosage forms in which metronidazole is released slowly from a drug carrier have been suggested as a means to reduce dosing frequency and systemic toxicity[7,8]. Nanotechnology involves fabrication of nanoscale structures which are observed visibly under high resolution. These molecular assemblies are especially designed for attaining their target functions[9]. Based on their critical packing parameter and hydrophilic lipophilic balance (HLB), these molecules are self-assembled to various morphologies including micelles, sheets and vesicles (liposomes, transferosomes, exsosomes, niosomes etc.) [10]. Furthermore, these vesicular formulations had been more exploited in the field of transdermal drug delivery[11]. They offer many advantages over conventional delivery systems like bio-compatibility, non-toxicity and ability to modify drugs' bioavailability[12]. In addition to the utilization of vesicular carriers for transdermal drug delivery, nanotransfection approaches (TNT) have been recently introduced for topical and controllable delivery of reprogramming factors across the skin. These approaches allow delivery of controlling factors by applying intense and

highly focused electric field using arrayed nano-channel. Hence, TNT can deliver the cargo to skin in rapid and non-invasive manner[13]. In this manuscript, the strategy of using transferosome s as a vesicular nano-carrier has been selected and investigated for efficient transdermal delivering of drugs and bypassing their oral problems. Transferosomes are ultra-flexible vesicles with a bilayer structure. They can penetrate the skin easily and overcome the barrier function by squeezing through the intracellular lipid of the stratum corneum[14]. After application of Transferosomes on the skin, they move from the dry stratum corneum to a deep hydrated layer according to the osmotic gradient. The presence of surfactant in their structure helps in solubilizing the lipid in stratum corneum and permits high penetration of the vesicles[15]. In the present investigation, we attempted to develop and optimize transferosomal gel containing metronidazole, for improved transdermal permeation.

MATERIALS AND METHODS

Materials

Metronidazole and Soya PC was purchased from Himedia Laboratory, Mumbai. Ethanol, chloroform and carbopol-934 purchased from CDH chemical Pvt. Ltd. New Delhi. Dialysis membrane of Mol Wt cutoff 1200 was purchased from Himedia Laboratory, Mumbai. Demineralized and double distilled water was prepared freshly and used whenever required. All other reagents and chemicals used were of analytical grade.

Methods

Determination of λ_{max} of metronidazole

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.4 pH buffer solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.4 pH buffer solution. Prepare suitable dilution to make it to a concentration range of 5-25µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). A graph of concentration Vs absorbance was plotted.

Preparation of metronidazole loaded transfersomes

Soya PC (0.5, 1.0, 1.5, 2.0% w/v) was dissolved in ethanol (5-25% v/v) and heated up to 30±1°C in a water bath in a closed vessel[16]. Distilled water or drug solution in distilled water (1% w/v solution), which is previously heated up to 30±1°C, was added slowly in a fine stream to the above ethanolic lipid solution with continuous mixing using a magnetic stirrer at 900 rpm. Mixing was continued for another 5 minutes and finally, the vesicular dispersions resulted was left to cool at room temperature (25±1°C) for 45 minutes.

Optimization of transfersomes formulation

Transfersomes formulation optimized based on the evaluation of mentioned strategy procedure resting on the source of average vesicle size and (%) entrapment efficiency (EE). In the transfersomal formulation, the ratio of lipid was optimized by taking their different ratio such as 0.5, 1.0, 1.5, and 2.0% w/v ratio and all other parameters were kept remain constant. the ethanol content was optimized by taking their different quantity such as 5, 10, 15 and 20 and all other parameters were kept remain constant. Drug concentration optimized by taking different concentration of drug such as 1, 1.5, and 2.0% w/v and prepared their formulation and all other parameters such as Soya PC, stirrer time kept remain constant. Stirrer time was optimized by stirring the formulation for different time, i.e., 5, 10, and 15 min.

Characterization of metronidazole-loaded transfersomes

Microscopic observation of prepared transferosomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared transferosomes formulation.

Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK).

Zeta potential

The zeta potential was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm.

Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded transfersomes dispersion was placed in the Ependorf tubes and centrifuged at 17000 rpm for 30 min. The transfersomes along with encapsulated drug were separated at the bottom of the tubes. Plain transfersomes without drug was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 278 nm.

Preparation of gel base carbopol

Carbopol 934 (1%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. Transferosomal preparation corresponding to 2% w/w of Metronidazole was incorporated into the gel base to get the desired concentration of drug in gel base.

Characterization of transfersomes containing gel

Measurement of viscosity

Viscosity measurements of prepared topical transfersomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10 rpm.

pH measurements

The pH of selected optimized formulations was established with the help of digital pH meter. The pH meter was calibrated with the help of buffer solution of pH 4, pH 7 and pH 9. After calibration, the electrode was dipped into the vesicles. Then, pH of selected formulation was measured and readings shown on display were noted.

Drug content

Accurately weighed 100 mg of topical transferosomal gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered by means of Whatman filter paper No. 1. Then, 1.0 ml of filtered solution was engaged in 10 ml capacity of volumetric flask; moreover, volume was ready up to 10 ml by means of methanol. This

solution was analyzed using UV spectrophotometer at λmax 278 nm.

Extrudability study

Extrudability was determined on the amount of the gel extruded as of collapsible tube on appliance of certain load. More the quantity of gel extruded shows better extrudability. It was determined by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadability

Spreadability of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. An apparatus in which a slide fixed on wooden block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadability, 2-5 gm of gel placed between two slides and gradually weight was increased by adding it on the weight pan and time required with the top plate to face the distance of 10 cm on adding 80 g of weight was noted. Good spreadability shows lesser time to spread. It is determine by formula given below[17].

$$s = \frac{m * l}{t}$$

Where, S=Spreadability (gcm/sec), m = weight tied to the upper slide (20 grams),

l= length of glass slide (6cms), t = time taken is seconds.

In vitro drug diffusion study

The in vitro diffusion study about is conveyed by utilizing Franz diffusion cell. Egg membrane is taken as semi penetrable membrane for diffusion[18]. The Franz diffusion cell has receptor compartment with an effective volume roughly 60 ml and compelling surface area of permeation 3.14sq.cm. The egg membrane is placed between the donor and the receptor compartment. A 2cm^2 size patch taken and weighed then set on one face of membrane confronting donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is encompassed through water casing to keep up the temperature at 32 ± 0.5°C. Warmth is furnished utilizing a thermostatic hot plate with a magnetic stirrer. The receptor liquid is mixed by Teflon covered magnetic bead which is put in the diffusion cell. Amid each testing interim, samples are pulled back and replaced by equivalent volumes of fresh receptor liquid on each sampling. The samples withdrawn are analyzed spectrophotometrically at wavelength of drug 278 nm.

RESULTS AND DISCUSSIONS

The absorption maxima of metronidazole were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer (Labindia UV 3000+) using concentration range of 5-25µg/ml metronidazole in 7.4phosphate buffers Figure1. Metronidazole showed a linear relationship with correlation coefficient of 0.999 in the concentration range of 5-25µg/ml in phosphate buffer pH 7.4. All the data of preformulation study were found similar as given in standard monograph which confirmed that the drug was authenticated and pure in form and it could be used for formulation development of metronidazole loaded transfersomes. Optimization of the transfersomes to generate the formulation code was done using the strategy as reflected in Table 1 optimization of lipid concentration, Table 2 optimization of ethanol concentration, Table 3 optimization of drug concentration and Table 4 optimization of stirrer time. It was observed that the vesicles dimension of transfersomes was increased with raising the concentration of phosphatidylcholine and ethanol. There was no noteworthy difference observed in average vesicle size with increasing the drug concentration, but with increase in the stirrer time the size of vesicle decreased from 145.56to 132.23after 15 min of stirring. Considering the EE, it was observed that the percent drug entrapment increased with escalating the concentration of ethanol and on escalating the time of stirring. The resulted formulation code F-14 was considered as the optimized formulation. The average vesicle size of optimized formulation (F-14) observed as 132.23 nm, zeta potential observed as -24.12mV and %EE was found as 71.12%. Prepared gel of transfersomes loaded with metronidazole (TG-14) was prepared and evaluated for viscosity, pH, % drug content, extrudability, spreadability and drug release study (Table 5). It was found that viscosity of prepared gel TG-14 was 3560cps, % assay was 98.89±0.45%, extrudability was 156g and spreadibility (g.cm/sec) was found that 11,23(g.cm/sec) respectively. In vitro drug release from Transfersomes was carried out using Franz diffusion cell method and found 85.56% in 12 hr. In first 30 min it was 22.2% drug release which slightly high. It was due to the release of free drug present in bag after leaching from Transfersomes. Drug release from transferosomal formulation was found in very sustained and controlled manner.

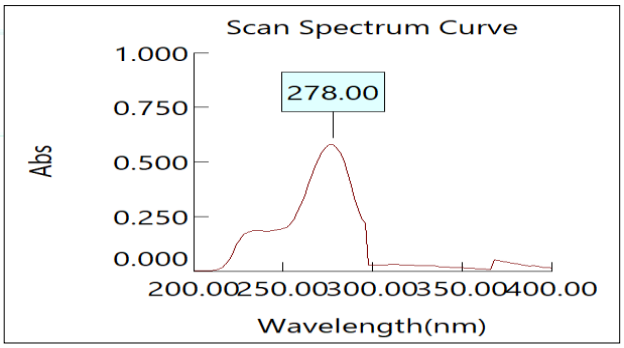

Figure 1 Wavelength maxima of metronidazole in phosphate buffer pH 7.4

Table 1 Optimization of lipid concentration

Formulation code	Soya PC (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F1	0.5	10	1.0	320.23	65.56
F2	**1.0**	**10**	**1.0**	**285.65**	**70.23**
F3	1.5	10	1.0	266.54	65.52
F4	2.0	10	1.0	295.56	48.89

Table 2 Optimization of ethanol concentration

Formulation code	Soya PC (% w/v)	Ethanol	Drug (% w/v)	Average vesicle size (nm)	% entrapment efficiency
F5	1.0	5	1.0	275.56	65.25
F6	**1.0**	**10**	**1.0**	**210.23**	**72.23**
F7	1.0	15	1.0	265.56	69.98
F8	1.0	20	1.0	274.45	65.23

Table 3Optimization of drug concentration

Formulation code	Soya PC (% w/v)	Drug (% w/v)	Ethanol (ml)	Average vesicle size (nm)	% Entrapment efficiency
F9	**1.0**	**1.0**	**10**	**210.12**	**78.25**
F10	1.0	1.5	10	265.56	65.23
F11	1.0	2.0	10	295.45	45.58

Table 4 Optimization of Stirrer time

Formulation code	Soya PC: (% w/v)	Drug (% w/v)	Stirrer time (min)	Average vesicle size (nm)	% Entrapment efficiency
F12	1.0	1.0	5	145.56	45.56
F13	1.0	1.0	10	165.58	62.23
F14	**1.0**	**1.0**	**15**	**132.23**	**71.12**

Table 5 Characterization of gel based formulation of prepared gel containing metronidazole loaded Transfersomes

Gel	Viscosity	Assay	Extrudability	Spreadability
TG-14	3560cps	98.89±0.45%	156g	11.23(g.cm/sec)

CONCLUSION

Transfersomes were prepared and optimized on the base of average vesicle size and % drug entrapment. The optimized formulation was further incorporated with gel base (Carbopol gel) and characterized for their viscosity, pH, % drug content, extrudability, spreadability and drug release study. The average vesicle size of optimized formulation (F-14) observed as 132.23 nm, zeta potential observed as -24.12mV and %EE was found as 71.12%. It can be concluded that prepared gel containing metronidazole -loaded transfersomal formulation was optimized and can be of use for topical preparation.

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